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UNITED STATES DEPARTMENT OF AGRICULTURE
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BUREAU OF ENTOMOLOGY AND PLANT QUARANTINE

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TITLE

UNITED STATES DEPARTMENT OF AGRICULTURE
Agricultural Research Administration
Bureau of Entomology and Plant Quarantine
Division of Golden Nematode Control

MANUAL OF SURVEY AND LABORATORY METHODS USED BY THE DIVISION OF GOLDEN NEMATODE CONTROL

Hicksville, L. I., New York

November 2, 1950

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FOREWARD

This manual has been prepared as a guide to supervisors and crew leaders who are often in need of a reference source. It is designed to disclose general information to new employees, and it is hoped that the same purpose might be served with those less intimately associated with the collection and processing of soil sample lots for the golden nematode. The procedures set forth in this manual have been tried and found to be satisfactory. However, they do not necessarily represent the only method. Through the efforts of our field workers, these methods and techniques are constantly being improved and it is hoped that all personnel will continue to submit their suggestions.

The assistance and suggestions given by the Division of Nematology, Bureau of Plant Industry, Soils and Agricultural Engineering, and The Department of Plant Pathology, New York State College of Agriculture, in the preparation of this manual are gratefully acknowledged.

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INTRODUCTION

The golden nematode (Heterodera rostochiensis Woll.) is a soil infesting parasite which attacks potato and tomato plants. It is known to have been present in England for more than 30 years. On the European continent this parasite occurs in Sweden, Denmark, Germany, and the Netherlands. It is primarily from experience in England that the destructiveness of this nematode is known. On some infested lands there potatoes cannot be grown profitably more than one out of eight years. The presence of the golden nematode in the United States was reported in 1941, following investigation of decreased potato yields reported by a farmer at Hicksville, Nassau County, New York.

The nematode lives on potato and tomato plant roots, from which it extracts the juices thereby retarding plant growth and reducing crop yield. Following penetration of the root epidermis for feeding purposes the posterior portion of the fertilized female larva containing the egg masses extrudes outside the root surface. It develops into a white, lustrous, spherical-like cyst which later changes in color to yellow or orange and subsequently to red or brown. The mature cysts are easily detached from potato roots and remain in the soil where they are difficult to detect. Cysts, or larvae therefrom, are easily disseminated by the movement of soil from infested fields and by natural means such as wind and waterflow. This nematode is not an internal parasite of potatoes, and its known ability to survive in the soil for periods ranging up to 8 years in the absence of its primary host makes control by crop rotation most difficult. Nematode population increases will occur in lands planted to potatoes for successive years without rotation and progressive reduction in crop yields may be expected. It is very important that this organism be recognized at an early stage before crop damage becomes apparent. The best method available for the detection of golden nematode is the collection of samples of soil from potato and tomato fields and the microscopic examination of wash residues of such samples.

SURVEY METHODS

The Survey Crew:

Personnel assigned to soil survey activities normally are divided into crews, each consisting of two or three men, one of whom is designated as the crew leader. The number of men assigned to survey a given property or area will vary in accordance with the district in which they operate and the scope of such operations. It will be found that on the average one man can survey 25 acres per day; therefore, the crew may be varied from one to several men depending upon the type and scope of survey to be conducted.

Equipment:

Each crew is provided with the following equipment: a map of the area to be surveyed; a supply of No. 12 wet-strength, double-thickness paper bags; a good quality of black, wax, marking crayon; a pointing trowel for each member of the crew; tape or twine for sealing the sample lots; a bristle brush for cleaning shoes and equipment; and essential forms on which to record operations. Each crew leader keeps a daily log or diary of his operations in a notebook which is provided for this purpose, and at the conclusion of the survey season these notebooks are to be returned to the headquarters' office.

Field Sampling:

Crews are assigned a district or section in which to work. Upon arrival at a premises subject to survey the crew leader looks over the land, determines the boundaries, size and shape of the field, and makes a mental notation of how he plans to survey the property. It has been found advisable to divide a property to be surveyed into several small working units. By making such subdivision it will be possible to return directly to a given block should infestation be found and will facilitate reinspection. It also permits a systematic survey of the field and gives the laboratory a soil sample lot that is of the proper size for processing. Normally on the initial survey the field is divided into working units of 3/4 acre to 1 acre. These blocks will be approximately 48 x 72 paces (3/4 acre), or if it is desired to work the field in areas of 1 acre the block is increased to 48 x 104 paces.

The crew leader paces the field to determine its length. Then he divides this length by 72 or 104, depending upon the size of block

he expects to work. Adjustments are made in the dimensions of the last block, or blocks, to care for any irregular edges to the field. Under normal conditions the initial survey is conducted by sampling each block in a grid pattern, collecting soil every 8 paces. This system is commonly referred to as the "8 x 8-pace method." The soil sample lots obtained weigh between 4 and 6 pounds each. For reinspection purposes, or when a field or portion of a field is under suspicion or shows symptoms of infestation, it is advisable to divide the field into smaller plots and to inspect it intensely on a 4 x 4 or a 2 x 2-pace pattern. In such cases, the block to be inspected is reduced to 24 x 48 paces for the 4 x 4 pace method and 12 x 24 paces when it is desirable to inspect plots at 2-pace intervals.

Assuming that the field is 210 paces long, the leader would start the first man on the edge of the field with bags numbered 1 through 3, the first two bags each to contain soil sample lots from a section of the row 72 paces long and the third bag from a section 66 paces long. The crew member starts up the edge of the field picking up about a tablespoonful of soil on a pointing trowel at intervals of 8 paces, putting it in bag No. 1, until he has gone up 72 paces. (See Figure No. 1) He then steps over 8 paces into the field at a right angle and sets bag No. 1 down. He comes back to the first row and resumes operations, using bag No. 2, until another 72 paces are covered. Again, he steps over into the field 8 paces, which places him in line with bag No. 1, and sets down bag No. 2. He returns to the spot where he left off on the original row and continues with bag No. 3. After 66 paces, with soil sample lots being placed in bag No. 3, he will come to the end of the field. He again steps over 8 paces along the back edge of the field. This should bring him in line with bags Nos. 1 and 2. He starts back toward the road from this point picking up soil every 8 paces until he reaches bag No. 2. Then he steps over 8 paces further into the field and sets down bag No. 3, returns and works bag No. 2 down to No. 1, etc. He continues this procedure until three return trips from the road to the back end of the field have been completed. On his last trip back to the road he notes that bag No. 3 is completed when he reaches bag No. 2. He simply folds the top of this bag which now contains a completed soil sample lot and carries it under his arm, and continues this procedure until he reaches the road. The leader lays the bags necessary for a tier every 48 paces along the front of the field. Therefore, when the first man completes his tier and reaches the road, he finds that the second man has started his tier just 8 paces over in the field from where he finished. He simply follows along the edge of the field until he comes to the next tier not being worked and begins again.

Collecting of Grader Samples:

Soil is secured from accumulations under the grader, under the loading belt, in storage bins, or in any location where potatoes are concentrated in quantities. Frequently farmers place grader debris in burlap bags, baskets, and other containers in the storage cellar for removal to the field and disposal. Other growers merely dump the grader debris in piles around the storage cellar. These sites are excellent sources of soil samples. It is found generally that all such debris has a high content of potato vines, sticks, stones, potato skins, and other extraneous offal. Care is taken to include as little of this type of debris as possible. In other words, as much soil as possible is secured. As debris is found in a number of locations in and around a storage cellar, dirt from each pile is included in the sample lot. In cases where large quantities of soil are available, it is desirable to obtain two or more sample lots. Each paper bag is filled to a depth of 4 to 5 inches, the top folded and sealed with tape. Every precaution is taken to avoid leakage of soil from the bags.

Plant Root Examination Method:

The method of examining potato roots may be used to advantage under certain conditions. The golden nematode does not cause malformation of the tuber, nor does it produce galls on the roots as is the case with certain other types of nematodes. The golden nematode attacks the root system, entering the roots as small eel-shaped larvae which become sedentary and then begin to swell. The female later protrudes from the surface of the root as a white spherical body. The color changes slowly to light yellow, to orange and then to a dark, golden brown, at which time the female drops from the roots and remains in the soil. The best time to examine by the plant-root method is while the nematode is in the white and orange stages. On Long Island, this is about the last two weeks in June. As a general guide it may be said that the cysts are in this stage when the potatoes begin to blossom. Fields are looked over carefully, and patches showing plants with weak, spindly stems and stunted tops are selected. Examinations also are made around buildings or where grading debris has been disposed of on fields. The plant is carefully removed from the ground with a pointing trowel. The roots are separated from the dirt until only the roots and a small amount of soil remain. No attempt is made to remove soil that is clinging to individual roots. Any unnecessary roughness causes the nematodes to fall from the roots. With the aid of a hand lens the root system is examined carefully for the presence of female cysts. Specimens collected in this manner are placed in vials containing formalin solution and referred for further laboratory study.

Other Surveys:

The collection of soil sample lots from nurseries, greenhouses, cold frames, plant beds, baggage, cargo and other items presents special problems. In all cases a systematic procedure should be followed, even though the method of obtaining the soil may vary. Obviously it would be impractical to survey a nursery on an 8 x 8 pattern basis. Therefore, the crew leader should divide the nursery into sections according to the type of stock grown or by following natural boundaries such as roads and walk-ways; then soil sample lots should be collected systematically through each given area with notations as to where each originates. This method will apply also to survey of greenhouses and other such surveys. The examination of products being shipped through the mails presents a different problem, and it may be necessary to wash the roots of an individual plant in order to determine whether golden nematode cysts are present. In some instances the collection of debris in the bottom of a packing case or bag may be the only material for examination.

Sanitation:

In consideration of the characteristics and potentialities of dissemination, every reasonable precaution should be taken to prevent the spread of this microscopic organism. Vehicles assigned to survey are not permitted to enter any property. They must remain on paved highways or recognized thoroughfares. Trowels must be free of soil-collecting recesses and grooves. A brush is provided for the purpose of cleaning the inspectors' shoes after leaving fields or storage houses. It is advisable for inspectors to wear trousers free of cuffs. Vehicles used on survey must be cleaned periodically by washing and must be free of soil at all times.

Labeling and Recording:

One of the most important steps to be taken is the proper labeling and recording of the soil sample lots. The bag should be labeled in a manner whereby all information is visible after the bag has been sealed. The inspector's collection number, which consists of his three initials and the number pertaining to that collection, is recorded on the top line of the bag. The first collection number made on the survey is No. 1. Each collection thereafter, regardless of the state or county, is numbered in a series. Thus the inspector's first collection appears as "JFS-1," the second collection as "JFS-2," etc. The name of the farmer, grading house, map designation or field number is recorded on the second line. The date (month and day only) is recorded in the lower left corner of the bag.

The sample lots secured on each collection in a given location are numbered in series beginning with No. 1. On the last sample in a series following the sample number the notation "End" is made. This identification series number is recorded in the lower right-hand corner. Thus the soil sample lots collected at a given location have a collection number supplemented by a series number, the latter on each new property starting with No. 1. The following is an illustration of the essential notations to be recorded on each sample lot collected:

JFS - 26
A. B. Jones
8/15 1

General field survey form GN-2 will be used. (See Figure No. 2) It is important that this form be filled out in its entirety, in duplicate, and that the information requested be stated clearly. If more than one page is used, in the upper right-hand corner of each page the number of pages in the report is indicated, such as "1 of 5 pages." On the first line there is stated the names of the state and county in which the work is conducted. If there is a map designation to the property this is noted also. The name of the farm operator and his mailing address are placed on the second line. Line 3 provides space for a brief description of the exact farm location. On line 4 is placed the crew leader's collection number, followed by the sample pattern (such as 8 x 8) and the number of acres inspected. The name of the crew leader and the inspectors who assist him and the date on which the inspection was completed are on line 5. If this is the initial survey of the property during the crop year, a check is made in front of the word "initial" on line 6. The blocks "confirmatory" and "delimiting" are reserved for properties on which infestation has been found. When survey is made for some special reason, such as nursery survey, the block marked "other" is used. Field survey crews will not check blocks marked "negative" or "positive."

In the column marked "Sample Lot No." soil sample lots collected, beginning with No. 1, are listed. Following the last sample lot number listed, a diagonal line should be drawn to the bottom of the page to indicate that nothing more follows. All other information requested on the face of this form is to be furnished by the laboratory processing the soil sample lots.

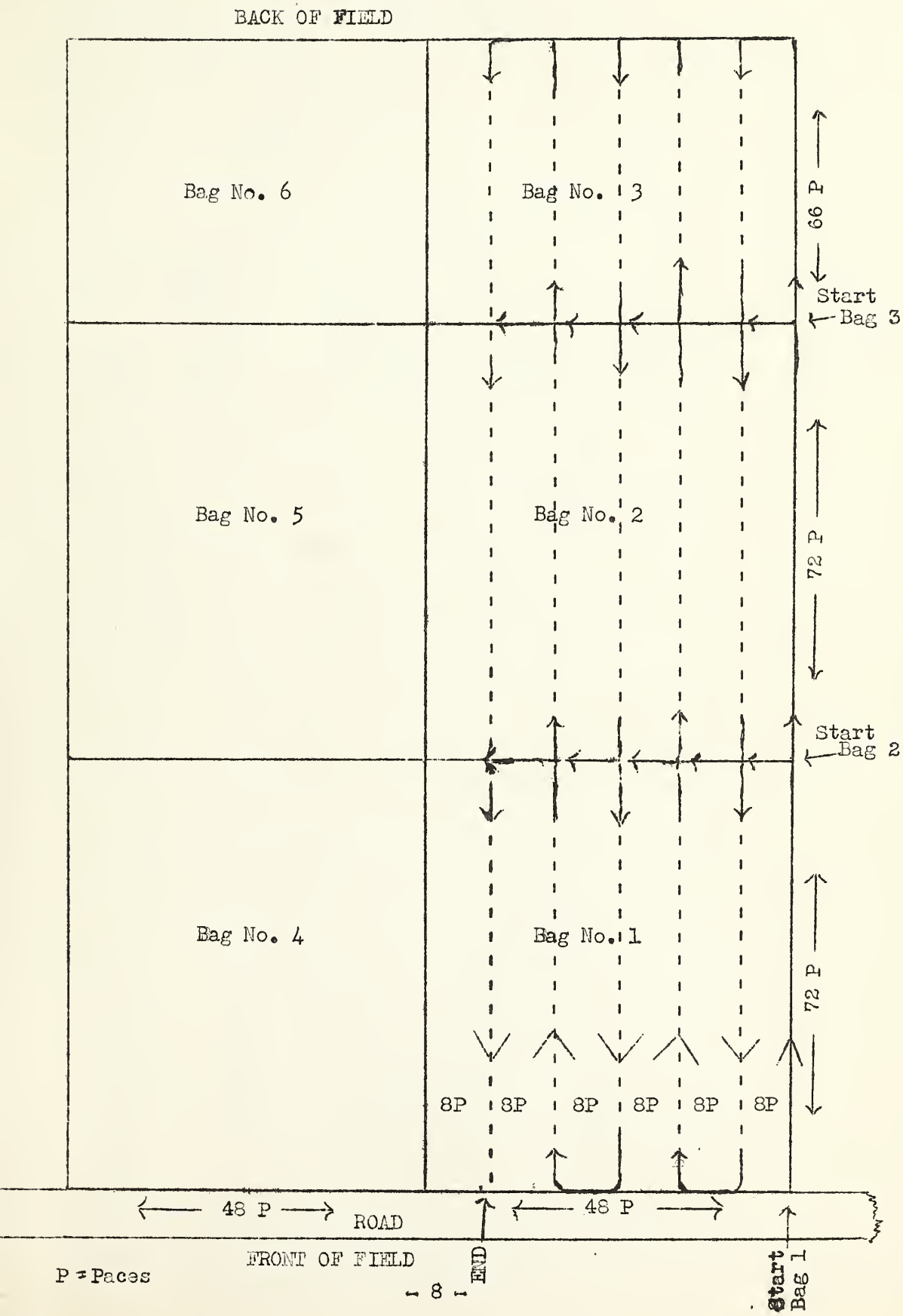
On the reverse side of Form GN-2 a simple diagram of the property inspected should be drawn. On this diagram is noted the location

of each soil sample collected and the dimensions of the block from which the sample is taken. Enough landmarks should be shown, together with names of roads, telephone pole, number, etc., so that the field may be relocated without difficulty. On the lower portion of the form the inspector indicates the type of crop on the field at the time it is inspected and other information, such as whether the crop has been harvested or not.

Form GN-2a is used for recording information relative to collection of grader samples. (See Figure No. 3) This report is also made in duplicate. On the first line the state, county, inspector's name and year are recorded. In column 1 there is placed the collection number; in column 2, the date of collection; in column 3, the operator's name; in column 4, mailing address of the operator; in column 5, farm storage house or packing house location; in column 6, total acres grown by the farmer (This information may not always be available, but if possible obtain the nearest approximate figure); in column 7, the approximate acreage of potatoes represented by the sample collected. (For example, if a farmer grows 50 acres of potatoes all stored in one cellar, and he is half through shipping when soil is secured from the 25 acres shipped, the entry in this column would be 25 acres.) In column 8 the number of samples collected at a given barn or warehouse are indicated. Columns 9, 10, and 11 are to be left blank, as well as the last line on the page. These entries are to be made by the laboratory personnel at the time the soil sample lots are processed.

Forms GN-2 and GN-2a, Field Survey Reports, together with a narrative of the week's operation, will be submitted in duplicate to the headquarters' office weekly.

Figure 1



P = Paces

Figure 2

GN-2 (Rev. 3/15/48) GOLDEN NEMATODE SURVEY 1 of 1 Pages

State New York County Nassau Map Designation 58-Z-9

Farm Operator John Doe Address 100 W. Main St, Ruralton

Farm Location N/E/cor West Main St. & First Ave.

Coll. No. RAD-50 Sample Pattern 8X8 Acres Inspected 19

Inspector(s) Dare, Jones, White Date 10/18/50

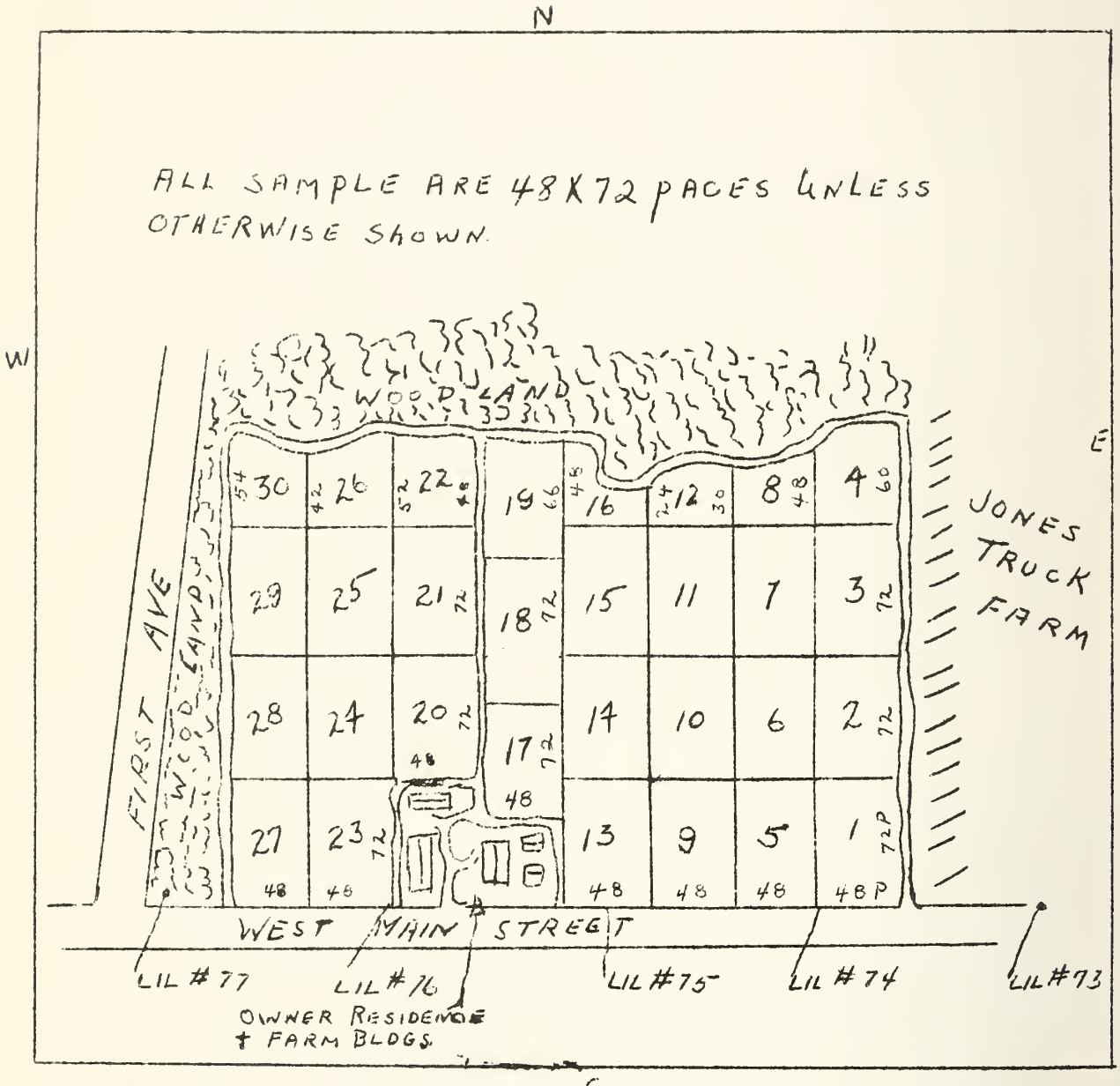
Survey: [✓] Initial [ ] Confirmatory [ ] Delimiting [ ] Other Negative [ ] Positive [ ]

Sample Lot No.	GN No. Cysts	GN Neg.	Iden. No. of Slides & Vials Filed	Sample Lot No.	GN No. Cysts	GN Neg.	Iden. No. of Slides & Vials Filed
1				17			
2				18			
3				19			
4				20			
5				21			
6				22			
7				23			
8				24			
9				25			
10				26			
11				27			
12				28			
13				29			
14				30			
15							
16							

Determined by _____ Date _____

Confirmed by _____ Date _____

Remarks _____



Type of crop on property and Estimated Acreage of Each 19A POTATOES

Status of Field at time of Survey PLANTED

Other Remarks

Figure 3

GN-2a (Revised 3/15/49)

GOLDEN NEMATODE SURVEY
GRADER AND SHIPPING POINT INSPECTION RECORD

State Virginia County Gooch Inspector(s) Jones Year 50

Coll. No.	Date	Operator	Address	Location	Total Potato A. Farmed	Approx. Pot. Acres Rep. By Samples	No. Samples	Neg.	No. Cysts	Iden. No. of Slides and Vials Filed
RAJ-8	7/16	E. K. Pomme Co.	Goochville, Va.	Goochville	Dealer	300	30			
		List of growers represented:								
		Jack Bryant, John Cotton Bill Carter, Wm. Trodoze								
RAJ-9	7/16	C. J. Atkitter	Goochville, Va.	RT 78 ½ mi S. of Hanover	300	245	20			
RAJ-10	7/17	O. K. Skoltivtch	Goochville, Va.	NE Cor. Int. of RT 78 & Rd 216	125	75	8			

Determined by _____ Date _____

LABORATORY METHODS

This section on the work of the laboratory is intended to outline a method of recovering golden nematode cysts from soil sample lots. It is based on procedures in operation at the laboratories of the Division of Golden Nematode Control. These procedures were developed around the fact that golden nematode cysts will float. This is particularly true when soil sample lots have undergone a period of dehydration. Hence, before processing, soil samples are stored in greenhouses on well ventilated racks. In order to make golden nematode determinations in a minimum amount of time and without sacrificing highly accurate results, there has been developed a two-phase processing cycle--a washing phase and an examination phase. By this means, laboratory personnel may alternate every two hours between washing and examining soil sample lots. The monotony of doing either phase for 8 hours each day is thereby relieved, and swift and accurate results are enhanced.

The need for infallible determinations has promoted important procedures aimed at entirely eliminating contamination possibilities. Neat work is required of established personnel and trainees: equipment is thoroughly washed after the processing of each soil sample; shelves and racks bearing samples are brushed and washed before reloading with new samples; and when infested material is found, sieves in use at the time are replaced until they can be thoroughly cleaned and inspected.

As in the case of every project, special problems have brought into being special equipment. Testing sieves currently in use are made by tinsmiths from medium-gauge tin. The finished product gives the appearance of a 4-inch-deep pan with a bottom made of 20- or 60-mesh brass screening. The bottom diameter of the sieve is 8 inches and the top diameter is $10\frac{1}{2}$ inches. For the purpose, such a sieve is superior to the factory-made, nesting type of testing sieve, in that it has a greater capacity, freedom of movement and is more rugged.

Special stands have been designed to hold testing sieves in place while material is being poured through. The stands are "L" shaped with the base made from a 1-foot section of 2 x 6 inch plank and an upright 1-foot piece of 2 x 4. To the upright section two heavy wire hoops are attached 3 inches apart. The lower hoop, which holds the 60-mesh sieve, is immovable and the upper hoop, which holds the 20-mesh sieve, is hinged so that it can be moved away in order to avoid interference with lower screen manipulation.

During the past year a soil washing machine has been designed which has shown encouraging results in recent sub-laboratory operations. Two machines are being used at the present time, and it is probable that at a future date improved machines will replace the manual method of washing soil samples.

On the following pages of this section, there are set forth detailed descriptions of the various phases of laboratory work and of the methods and techniques employed.

Washing Procedure:

Equipment: Three white enameled pails, 2 testing sieves (a 20-mesh sieve for screening out coarse material to be discarded and a 60-mesh sieve which will retain cysts that may be present), 1 wash-stand to support the 20-mesh sieve just above the 60-mesh sieve.

Purpose: To retain all of the floating material present in a soil sample lot for microscopic examination.

Technique: Step 1. A soil sample is placed in one of the enameled pails and roiled with a full force stream of water from a 3-foot hose. All material that floats is poured into a second bucket and allowed to stand while the initial pail is being re-filled by the roiling method. Again the first bucket is decanted, this time into a third pail. The need for additional washing of the soil in the initial pail may be ascertained by observing whether particles of floating material are clinging to the sides of the bucket. If further washing is indicated, the bucket is filled and emptied into the second pail, following "Step 2."

Step 2. The contents of the second pail, minus soil which has settled to the bottom, is poured through the testing sieves. This pail is re-filled in order to collect the floating material which was left after the first pour-off.

Step 3. The same treatment is given the third bucket. The alternating process of filling and decanting continues until it is judged that all floating material has been removed from the soil sample. The coarse material caught in the 20-mesh sieve is discarded. The material retained in the 60-mesh sieve is washed and transferred into a 600 ml. beaker, which has been labeled with the collection number and the soil sample lot number.

It is important for the washer to remember that soil from different areas varies markedly in the amount of organic matter present. Therefore, a definite amount of washing per sample cannot be fixed. In general, the procedure as described may be taken as the minimum amount of washing necessary. Further washing is left to the good judgment of the individual. A complete washing cycle includes thorough cleaning of all equipment.

Examination:

Equipment: Microscope (binocular with 15-18 magnification), small 80-mesh sieve (about the size of muffin tins), syracuse dishes, scalpels, forceps, needles, medicine droppers.

Purpose: To recover, for identification, cysts of the golden nematode that may be present in the soil sample.

Technique: Floating material which has been transferred into 600 ml. beakers by the washers is poured through the small 80-mesh sieves. While pouring, the beaker is rotated in such a way as to clear the sides of the beaker of material which adheres. Material thus obtained is apportioned into syracuse dishes. Sufficient water is added to float the material at a level near the brim. The correct amounts of material per dish will give a single, solid layer of material to be examined. The syracuse dishes are marked with a red line in order to identify the point from which examination commences. With the field of view focused on this point, the dish is revolved at a rate which will allow the eye sufficient time for examination of the material before it passes out of view. When the starting point (the red line) re-appears, another inner revolution is made to insure examination of portions of the material excluded from vision up to this point. Then the area in the center of the dish is included using a criss-crossing movement. Upon completion of the examination, all equipment is thoroughly washed so as to eliminate the possibility of contaminating the sample to follow. Suspect cysts are transferred by the examiner to frosted dishes and properly identified with collecting number and sample lot number.

Preparation of Microscopic Slides:

Equipment: Glass slides with one end frosted, number 1 thin cover glasses, dissecting needles and scalpel, clearing fluid[1], water, clearcol, slide labels, small brush, material for ringing slides[2], and ringing stand.

- -

1. Latco-Phenol Mounting Medium (clearing fluid)
   60 cc Phenol
   46 cc Lactic acid
   96 cc Glycerine
2. Materials for ringing slides:
   A - Clearcol, for ringing lacto-phenol mounts
   B - 50% parrafin plus 50% vaseline for the first ring on formalin mounts
   C - Zut (use acetone to thin), for second ring on formalin mounts

Purpose: To identify nematode cysts. The slide is filed for future reference and will serve as a record of infestations.

Technique: First, there is written on the frosted end of the slide the collection number and sample number from which the cyst is recovered. A very small drop of water is placed on the slide just to the right of center. With the cyst then transferred into the water, the posterior 1/3 of the cyst is cut off. This section is the most important and should show the following identifying characteristics: vulva, anus, punctation and/or pattern. Next, the 2/3 section is cut in half, care being taken to leave the neck of the cyst intact on one of the sections. All larvae and eggs are now removed from the sections by gently scraping with a needle. In cases where only eggs are present, larvae may be released by applying pressure on the eggs. The presence of larvae on a slide is very important for further identification and for viability determinations.

The three sections are arranged in a row fairly close together with the exterior surface up. A drop of clearing fluid is added and spread over an area about the shape and size of the cover glass. Arrangement and position of the sections are checked and the cover glass applied. The slide is ringed with clearcol, a small brush and a ringing stand being used for this purpose.

Finally, a gummed label is placed on the left end of the slide (opposite the frosted end) and the following information, reading from top of the label to the bottom, is recorded: slide number, map designation or port number, identification (i.e. "H. rost." if the cyst was viable and "NVHR" if the cyst was not viable), initials of the examiner, and date. Data required to be shown on slide are as follows:

Slide No._____	Collection No. _____
Map No. _____	Sample No. _____
Identification_____	Date_____
Initials_____	

Slides on all finds are sent to Beltsville for authoritative identification by nematologists. If possible, two slides are made, one for our files and one for Beltsville. All slides sent to Beltsville

are recorded on the laboratory form labeled "Record of Slides Sent to Beltsville." Slides to be mailed are wrapped in cellophane and placed in the wooden slide holders. Forms to accompany the slides are prepared at the office. Form EQ-449 is used for all specimens except those taken from Foreign Plant Quarantines material. In the latter case Form EQ-9 is used.

Preparation of Vials:

Equipment: Five percent formalin, small shell vials, bottles and stoppers, cotton, labels, forceps.

Purpose: To preserve and make record of cysts occurring in quantity.

Technique: The small shell vial is filled with 5% formalin about 2/3 full. The following information is recorded on a long slip of paper.

Coll. No. _____, Map No. _____, Date _____ Vial No. _____, Sample No(s). _____ No. of cysts _____

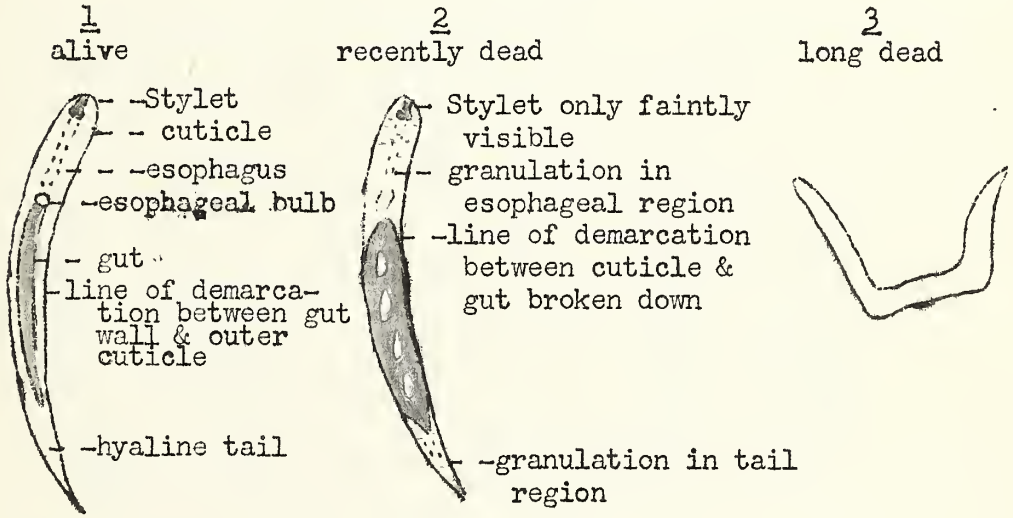
Cysts are transferred into the formalin contained by the shell vial and the vial is stoppered. The above slip of paper is placed in the bottle and cotton added. The shell vial is placed on this cotton in the bottle and cotton is packed around and above the shell vial. A stopper is placed in the bottle and an appropriate number on the stopper. The bottles are now numbered consecutively and are filed in groups of ten. The numbers should be recorded on the laboratory logs and the GN-2 forms.

Viability Determination:

It is of the utmost importance that viability of cysts be made with a great deal of accuracy since the action taken by quarantine officials will be dependent upon whether a cyst is viable or non-viable. It is important, therefore, that the individual preparing slides of such cysts have criteria upon which to base viability determinations. Limitations necessarily exclude extensive, time-consuming methods of making the determinations. The process must be as simple as possible, so that viability can be decided while the slide is being prepared. The following outline, as prepared by representatives of the Division of Nematology, may be used as a guide.

Outline

Figure 4



1. Stylet plainly visible
   Esophageal area - hyaline, clear, structures visible
   Gut full--i.e., black
   Line of demarcation between cuticle and gut wall
   No kinks, may or may not be movement
   Posterior area clear

2. Stylet only faintly visible or not visible
   Esophageal area cloudy, brownish tinge

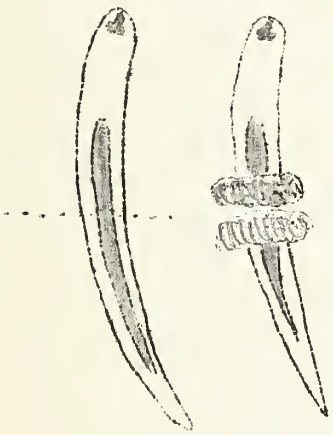
2. (Continued)

Gut either empty or vacuolated--granulation
(Perfectly healthy larvae use material in the gut as food if they cannot reach food. As this stored food is used, vacuolation may occur in the mass and if all the stored food is used, a viable larva may have an empty gut.)
May or may not be kinks, no movement
Tail area granular

3. Kinks in larvae vary in intensity and denote dead larvae.

Viable larvae have turgor (See Figure 5): non-viable larvae do not have turgor (See Figure 6). Therefore, if a larva is cut transversely with a needle or knife, the contents of the viable larva will "mushroom"; the non-viable larva will show no visible reaction to cutting.

Figure 5



viable

Figure 6

non-viable

Movement can often be detected by placing a bamboo sliver across the larva at a nerve center around the region of the bell, then gently massaging with a rolling motion.

Guide for Training New Personnel:

Equipment: Cyst-infested soil, syracuse dishes, needles, forceps, microscope.

Note: Neatness must be stressed in order to avoid bad habits which might later result in contamination.

Procedure: The suggested procedure may be broken down into four steps as follows:

Step 1. Important external identification characteristics are listed and explained to the trainees. They are:

1. COLOR . . is important in that it is the characteristic that first catches the eye and initiates further examination of a suspect object. The color range is from yellow to dark bronze.

2. SHAPE . . typically, the cyst of the golden nematode is spherical or orange shaped.

3. SIZE . . is a comparative characteristic--that is, actual dimensions are not measured but rather a cyst is compared with other objects that will at first confuse the prospective inspector. Microcysts are usually smaller than golden nematode cysts. Some of the seeds and seed pods are much larger in comparison.

4. TEXTURE OF CYST WALL . . Suspect material will show different reactions to pressure from a needle. This technique is called the "feel of the needle", and it is acquired only through handling of the material. Microcysts, for example, will usually break like an egg shell when needle pressure is applied. Many seeds will feel hard like stone. Seed pods are usually tougher than the cyst wall of a nematode. The latter will yield to pressure without breaking or tearing.

The prospective inspector then becomes acquainted with cysts by having to pick out specimens from infested material. Later, the objects he picks out are checked by an authority who demonstrates differences between the golden nematode cyst, microcyst, seed, seed pods and H. wiessi (a nematode commonly found in material). The trainee breaks and examines contents of some cysts.

Step. 2. This exercise should be initiated as soon as the trainee shows signs of being able to identify golden nematode cysts from other forms. Three syracuse dishes are set up using examined material which has been discarded by regular laboratory personnel. About six golden nematode cysts representing the different ranges in color, shape, and size which are ordinarily encountered in routine inspections are placed in the dishes which are numbered 1, 2, and 3. The trainee finds and transfers golden nematode cysts to a drop of water in a frosted dish. Specimens which he has identified as golden nematode cysts are then checked. As soon as he consistently recovers golden nematode cysts only, it is evident that at this point he recognizes cysts of this species when he sees them.

Step 3. It should be made certain that the trainee is observing the entire area of each dish. The size of a microscope field divides the area to be examined into three sections: A, B, and C (See Figure 7). Shaded circles represent the area covered by a single microscope field. One revolution of the dish from each microscope field results in the sections A, B, and C.

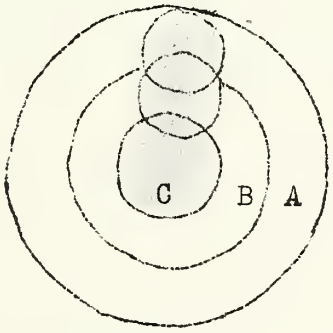


Figure 7

Figure 8 shows a typical test using the three dishes and six golden nematode cysts. Black dots represent golden nematode cysts.

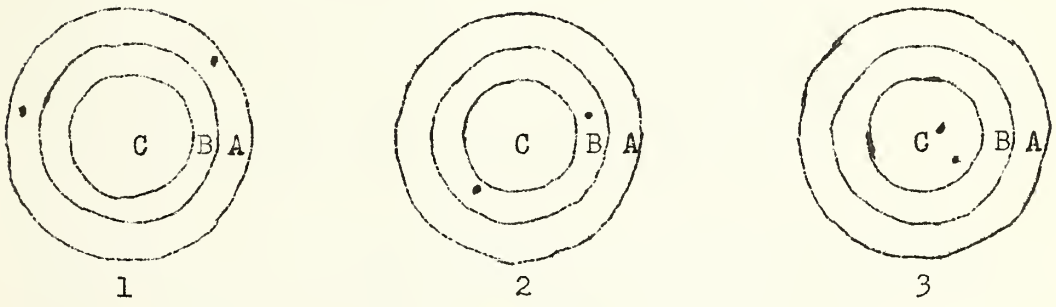


Figure 8

Using this system, section "A" is checked as indicated in dish 1; section "B" in dish 2; and section "C" in dish 3. Thus, areas missed by the trainee are disclosed and corrective measures can be taken.

Step 4. When the preceding exercise is completed in a satisfactory manner and in a reasonable length of time per try, it is well to increase the knowledge and interest of the trainee through means of photographs, literature and slides. (See page 22).

HETERODERA ROSTOCHIENSIS (Woll.)

Distinguishing characters used in identification of cysts:

Vulva – Rounded, not greatly protruding, but more or less on a level with the cyst wall.

Anus – Distinct, but very small in comparison to size of vulva.

Cyst wall – With open, runic pattern, and with punctations in more or less regular, horizontal rows.

Larvae – With rather weak knobs.

Egg – With plain shell – not punctate.

Larvae and eggs, when present, well protected within cyst wall.

Laboratory Reports:

Original copies of GN-2 forms prepared by field crew leaders reach the laboratory through the headquarters' office. These forms concern the laboratory in the following manner.

In case no golden nematode cysts are found, the third column marked "Neg." is checked for each soil sample listed. The block in the upper right corner is also checked as "negative" and the GN-2 is signed and dated by the laboratory supervisor on the line marked "Determined by" at the bottom of the page.

In the event that golden nematode cysts are found, the block marked "Positive" is checked with a red pencil. Each positive sample lot is also checked in red and the second column headed "No. cysts" is completed, as well as the fourth column which will show the file number of all slides prepared, any vial numbers and whether the cysts were determined as viable or non-viable. (See Figure No. 9) The notation "H. rost." after a slide number denotes a viable cyst, and non-viable cysts are indicated by the letters "N.V.H.R." following the slide number.

Five copies of EQ-449 forms (See Figure No. 10) are prepared for each slide or vial to be sent to the Division of Nematology. Information called for on these forms is taken from the GN-2 form. One carbon is retained in a pending file and dated; the other forms are submitted with the specimen.

GN-2a forms are completed by the laboratory by filling in columns 9, 10, and 11 in the same manner as was followed with GN-2 forms. They are signed and dated by the laboratory supervisor.

A laboratory log should be maintained as a record for each sample processed. (See Figure No. 12)

Completed forms GN-2 and GN-2a and a narrative of laboratory activity prepared on form GN-8 will be submitted to the headquarters' office weekly.

Reference Material on *Heterodera rostochiensis* (Woll.)

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Chitwood, B. G., et al. *Heterodera rostochiensis*, the golden nematode of potatoes, in New York State. Plant Disease Reporter 26: 390-391, 1942.

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Chitwood, B. G. and Feldmesser, J. Golden nematode population studies. Proceedings of the Helminthological Society of Washington 15 2: 43-55, lab-1-6, fig. 1-5, 1948.

Cobb, N. A. A new parasitic nema found infesting cotton and potatoes. Jour. Agr. Res. 11; 27-33, 1917.

Cunningham, H. S. and W. F. Mai. Nematodes parasitic on the Irish potato. Cornell Extension Bull. 712: 1-23, May 1947.

Filipjev, I. N. and J. H. Schuurmans Stekhoven Jr. A manual of agricultural helminthology. E. J. Brill, Leiden, Holland, 1941.

Goodey, T. Plant parasitic nematodes and the diseases they cause. Methuem & Co. Ltd., London, pp. 127-191, illustrated, 1933.

Johnson, L. R. and H. W. Thompson. Potato root eelworm in Yorkshire. Jour. Min. Agri. (Gr. Brit.) 52: 266-270, 1945.

McCubbin, W. A. Present status of golden nematode of potatoes. The American Potato Journal, Vol. 25, No. 4, pp. 131-133, April 1948.

McCubbin, W. A. Plant pathology in relation to Federal Domestic Plant Quarantines. The Plant Disease Reporter, Supplement 191, pp. 80-81, May 1, 1950.

Mai, W. F. Studying the golden nematode disease. Farm Research XIV(2), April 1948.

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Newhall, A. G. Golden nematode of potatoes jumps the Atlantic. Farm Research, July 1946, pp. 6-7.

Peters, B. G. Nematodes as crop parasites. World Crops 2(1): 11-15, January 1950. Leonard Hill, Ltd., 17 Stratford Place, London W. I., England.

Petherbridge, I. R. and W. A. R. Weston Dillon. Potato root eelworm. Jour. Min. Agr. (Gr. Brit.) 52: 264-265, 1945.

Popham, W. L. The golden nematode invades New York. Smithsonian Report for 1948: 349-354, 2 plates. Pub. 3969. U. S. Govt. Printing Office, 1949.

Staniland, L. N. Potato root eelworm in the southwest. Jour. Min. Agr. (Gr. Brit.) 52: 538-540, 1946.

Figure 9

GN-2 (Rev. 3/15/48) GOLDEN NEMATODE SURVEY 1 of 1 Pages

State New York County Nassau Map Designation 58-2-9

Farm Operator John Doe Address 100 W. Main St. Ruralton

Farm Location N/E Cor West Main St. & First Ave.

Coll. No. RAD-50 Sample Pattern 8X8 Acres Inspected 19

Inspector(s) Klaus, Jones, White Date 10/19/50

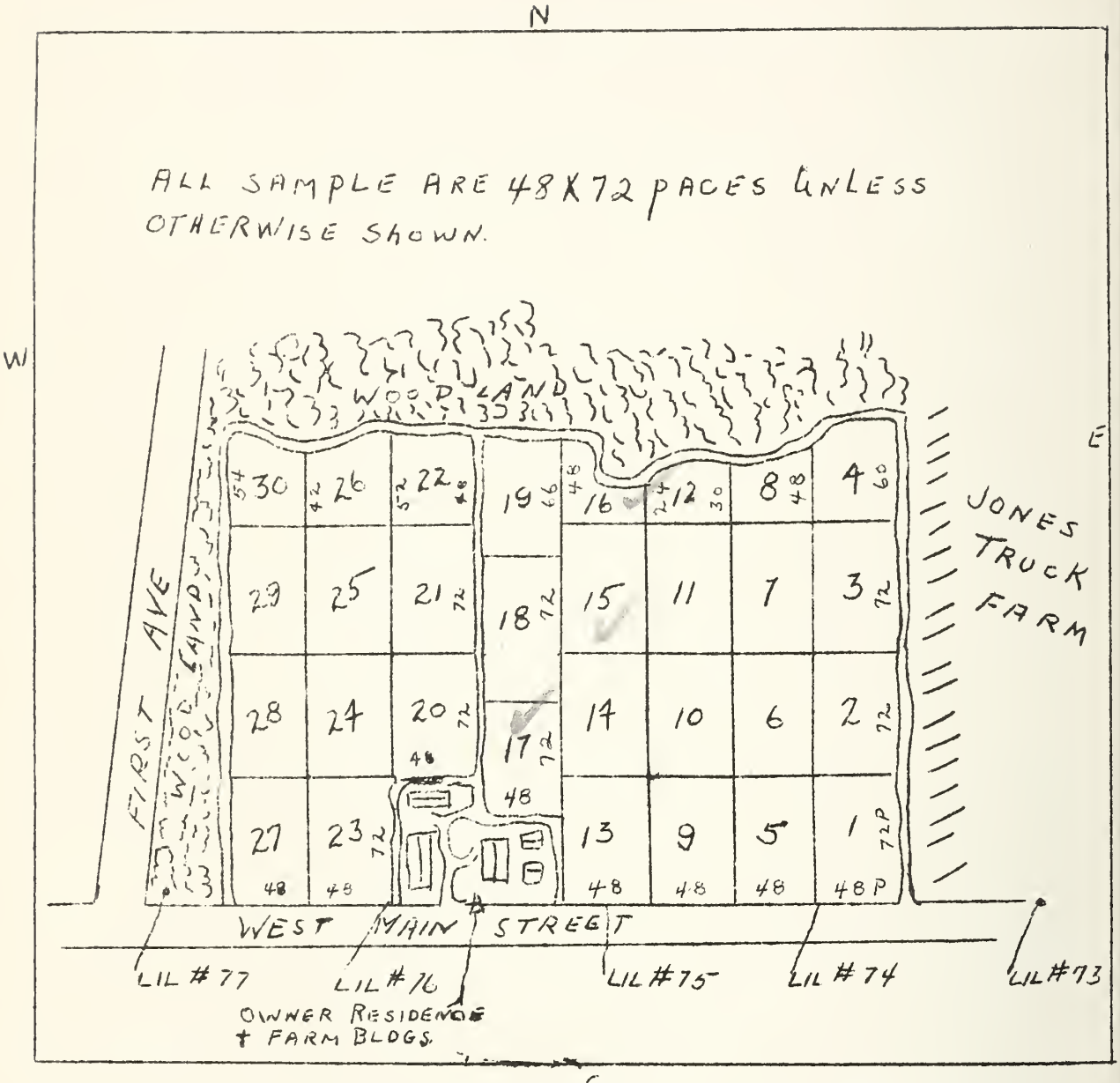
Survey: [✓] Initial [ ] Confirmatory [ ] Delimiting [ ] Other Negative [ ] Positive [✓]

Sample Lot No.	GN No. Cysts	GN Neg.	Iden. No. of Slides & Vials Filed	Sample Lot No.	GN No. Cysts	GN Neg.	Iden. No. of Slides & Vials Filed
1		✓		17	24		17-17-20 H. Rost. 23 cysts in Vial 57
2		✓		18		✓	
3		✓		19		✓	
4		✓		20		✓	
5		✓		21		✓	
6		✓		22		✓	
7		✓		23		✓	
8		✓		24		✓	
9		✓		25		✓	
10		✓		26		✓	
11		✓		27		✓	
12		✓		28		✓	
13		✓		29		✓	
14		✓		30		✓	
15	8		B1-2.0-17 H.V.H.R 7 cysts in Vial 50				
16	5		Vial 50				

Determined by H. O. Betz Date 10/30/50

Confirmed by _____ Date _____

Remarks _____



Type of crop on property and Estimated Acreage of Each 19A POTATOES

Status of Field at time of Survey PLANTED

Other Remarks

Figure 10

Form EQ-449
Revised 7/1/36

U.S. DEPARTMENT OF AGRICULTURE
Bureau of Entomology & Plant Quarantine

SPECIMENS FOR DETERMINATION

Collection No. J.F.S. 35

Owner John Doe

Address 100 W. Main St., Hicksville, N.Y.

County Nassau

Location N/E Cor. W. Main St. & 1st. Ave.

Location in grove or field Sample lot # 15

Host Plant Potatoes

Collector J. F. Spears

Date of Collection 9/18/50

Other information: Slide # R1-20-17 NVHR
R-17-20

Referred for identification to Dr. G Steiner, Nematologist

Referred by Dr O. Betz

Determined as H. Rostochiensis

Determined by G. D. Cobb
Div. Nematology

Notes:

(Send three copies of
this form with specimens)

Figure 11

GN-2a (Revised 3/15/49)

GOLDEN NEMATODE SURVEY

GRADER AND SHIPPING POINT INSPECTION RECORD

State Virginia County Gooch Inspector(s) Jones Year 19 50

Coll. No.	Date	Operator	Address	Location	Total Potato A. Farmed	Approx. Pot. Acres Rep. By Samples	No. Samples	Neg.	No. Cysts	Iden. No. of Slides and Vials Filed
RAJ-8	7/16	E. K. Pomme Co.	Goochville, Va	Goochville	Dealer,	300	30	V		
		List of growers represented:								
		Jack Oliver, Bill Carter, John Cotton, Williams, Rodoze								
RAJ-9	7/16	C. J. Atkitter	Goochville, Va	RD 78 1/2 mi. S. of Hanover	300	245	20	V		
RAJ-10	7/17	O. R. Skoltivitch	Goochville, Va	N.E. Cor. Int. of RD 72 + Rt 216	125	75	8	V		

Determined By O. O. Betz Date 7/30/50

Figure 12

GN-4 LABORATORY LOG Page 1

Examinations - G. N. Survey - 1950

Coll. No.	Bag No.	Washer	Examination Date	Examination By	Susp.	No. Cysts	Ident. As	Determined Neg.	Determined Pos.	Slides Vials Filed
VAL-75	1	Raw	11/9	JG	15	14	GN		✓	82-26-18 13 CYSTS-VIAL 20
	2	Raw	11/9	JG	0	0		✓		
	3	Raw	11/9	CRB	0			✓		
	4	Smith	11/9	CRB	0			✓		
	5	Smith	11/9	RMR	0			✓		
	6	Smith	11/9	RMR	0			✓		
	7	West	11/9	WAA	0			✓		
	8	West	11/9	WAA	0			✓		
	9	West	11/9	WSC	0			✓		
	10	West	11/9	WSC	0			✓		